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Research Article

The Taxonomy and Variation of Leaf Anatomical Characters in the Genus *Aegilops* L. (Poaceae) in Iran

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Abstract: This study concerns the evaluation of leaf anatomical characters (7 qualitative and 12 quantitative characters) among 26 accessions of the *Aegilops* L. species. The results of this study showed that the anatomical characters have high variation in the width of stomata, the number of long cells in the adaxial epidermis, the number of short cells in the abaxial epidermis, the number of stomata in the abaxial epidermis, the occurrence of prickles in the adaxial epidermis, and the width of phloem cells. The variations in anatomical characters were related to the cold and warm areas of Iran and commonly occurred among the tetraploid species. The indumentum and length of hairs in the epidermis, the occurrence and length of prickles in the epidermis, the width of sclerenchyma strands, and the number of stomata were found to be distinguishing characters among the species and of taxonomic value. Finally, *Ae. columnaris* Zhuk. is separated from both *Ae. umbellulata* Zhuk. and *Ae. triuncialis* L, whereas *Ae. speltoides* Tausch. can be considered a species different from *Ae. triuncialis*.

Key Words: Anatomical, *Aegilops*, taxonomy, Iran, variation

Introduction

The genus *Aegilops* L. (Family: Poaceae; Tribe: Triticeae) has 13 species in Iran (Bor, 1968, 1970). The life form of *Aegilops* species is annual with a mainly outcrossing breeding system (Waines & Barnhart, 1992). This genus is a pastoral plant that consists of only one donor species to the gene pool of *Triticum* L. (Lange & Jochemsen, 1992a) and causes the evolution of hexaploid wheat (Waines & Barnhart, 1992; Slageren, 1994). The ploidy levels of *Aegilops* species are diploid (2n = 2x =14, x = 7), tetraploid (2n = 4x = 28, x =7), and hexaploid (2n = 6x = 42, x = 7) (Bor, 1968; Lange & Jochemsen, 1992a, 1992b). It possesses wild species along with native races (Lange & Jochemsen, 1992a, 1992b).

There has always been debate among researchers concerning the taxonomy of the genus *Aegilops*. The complexes that occurred among the species were due to the high similarity and the hybridization between the *Aegilops* species, which causes high morphological similarity among these species (Lange & Jochemsen, 1992a). These findings have obscured the morphological limits of the species, and caused taxonomic confusion about the number of species and nomenclatural debates in the genus *Aegilops* (Morrison, 1993a, 1993b). Additionally, high levels of variation have been observed in this genus (Waines & Barnhart, 1992). Therefore, the genus *Aegilops* has held considerable interest among plant taxonomists (Keshavarzi & Rahiminejad, 2003).

Dube & Morisset (1987) reported that some of the significant morphological and anatomical characters can display the taxonomic position and the percent of similarity in Poaceae, especially in *Aegilops* species (Dube & Morisset, 1987; Baum & Gupta, 1990; Webb & Almida, 1990; Jarves & Barkworth, 1992). Dube & Morisset (1987) and Jarves & Barkworth (1992) illustrated that the occurrence of sclerenchyma and bundle sheaths (Kranz sheath), the width of sclerenchyma, the indumentum of leaves, and the length

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and frequency of epidermal hairs are important features that can identify relationships among the genera of Poaceae.

Kaminski et al. (1990) mentioned that the tetraploid species of *Aegilops* have higher variation in anatomical characters than the diploid species; however, the frequency of stomata among the diploid species is higher than that of the tetraploid species (Aryavand et al., 1999). Ecologically, these characters, along with the number of vascular bundles and the form of transection of the leaves, have varied in different regions (Dube & Morisset, 1987). Due to the wide range of variation, it seems necessary to evaluate taxa of the genus *Aegilops.* Regarding incomplete anatomical studies among *Aegilops* species in Iran, the necessity for biosystematic investigations seems very important.

The aims of this study were to determine the variation in leaf anatomical characters among different ploidy levels, to display the best anatomical characters for differentiating *Aegilops* species, and to determine the taxonomic relationships among these species.

Materials and Methods

In this study, *Aegilops* species were collected (7 species, 26 accessions) from natural habitats in Iran (central, west, south-west, north-west, and south-east regions) in July and August of 1999 and 2000 (Figure 1 and Table 1). The voucher specimens were deposited at the herbarium of Isfahan University. All the accessions were grown in the research field at Isfahan University in October 2000. Then, the transections of the leaves were prepared (Esau, 1967; Ogundipe & Olatunji, 1992) and 7 qualitative and 12 quantitative leaf anatomical characters, along with the matrixes of the characters and species (Tables 2 and 3), and the list of quantitative and qualitative characters (Tables 4 and 5) were studied with an Olympus Microscope, No. BX51. Statistically, estimation of the coefficient variation (C.V.) and standard deviation (S.D.) displayed the variation in anatomical characters. In order to determine the taxonomic status of *Aegilops* species, cluster analysis using a Euclidian distance coefficient, principle component analysis (PCA), and discriminate analysis were performed based on quantitative anatomical characters with SPSS 10.0.5 software (Table 7).

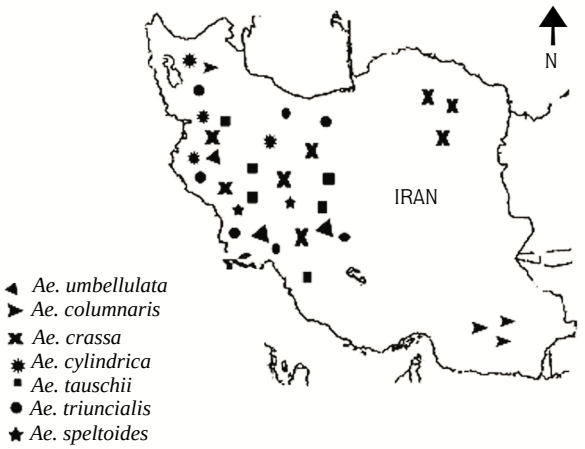

Figure 1. Distribution of the *Aegilops* species in natural habitats of Iran.

Results

The results of this study showed that 7 *Aegilops* species were mainly restricted in central, north-west, west, south-west, and south-east Iran (Figure 1 and Table 1).

The anatomical characters of the leaves and epidermis revealed that the width of phloems varied among these species (range: 17-58.7 µm) and the maximum value was found in *Ae. columnaris* Zhuk. (in the north-west), while the minimum belonged to *Ae. tauschii* Coss. (central Iran) (Figure 2 and Table 2). Among *Ae. triuncialis* L., *Ae. crassa* Boiss. (west), and *Ae. speltoides* Tausch. (south-west), the width of the xylem was 17-30.6 µm (Figure 2 and Table 2). In the adaxial epidermis, the width of stomata varied greatly among these species (7.82 µm for *Ae. cylindrica* Host and 27.8 µm for *Ae. columnaris*) (Figure 3 and Table 2). Moreover, the mean number of long cells varied between 162.24 (*Ae. tauschii*) and 790.74 (*Ae. cylindrica*). In the abaxial epidermis, the greatest length and width of stomata were 55.08 µm (*Ae. triuncialis*, south-west and north-west) and 27.6 µm (*A. tauschii*; west and centre), respectively (Table 2). The mean number of short cells in the abaxial epidermis varied widely between 16.5 (*Ae. crassa*) and 40.02 (*Ae. speltoides*) (Table 2). The mean number of stomata in the adaxial epidermis was 20.72 (*Ae. triuncialis*), while in the abaxial epidermis it was 48.76 and 48.7 for *Ae. tauschii* and *Ae. speltoides*, respectively (Table 2).

The indumentum of the epidermis (hair and prickle) is very different among these species, but *Ae. columnaris*

Table 1. Locations of the *Aegilops* species in natural habitats of Iran.

Species	Locality	Alt. (m)	Date	No. of population
Ae. speltoides Tausch. (2n = 14)	Chaharmahal & Bakhtiyari, Cheshme Ali	2080	2000	50
Ae. speltoides	Lurestan, toward Yasouj	2050	1999, 2000	51
Ae. tauschii Coss. (2n = 14)	Tehran, Delijan	1200	2000	70
Ae. tauschii	Tehran, Saveh	-	2000	71
Ae. tauschii	Lurestan, Khoram abad	1820	2000	72
Ae. umbellulata Zhuk. (2n = 14)	Lurestan, Khoram abad	1820	2000	52
Ae. umbellulata	Kohkiloye & Boyer Ahmad, Yasouj	2050	2000	54
Ae. umnellulata	Chaharmahal & Bakhtiari, Shahrekord	2000	2000	53
Ae. columnaris Zhuk. (2n = 28).	West of Azerbayijan, Oroomieh	1995	1999	265
Ae. columnaris	Systan & Balouchestan, Zabol	1250	1999	266
Ae. crassa Boiss. (2n = 28)	West of Azerbayijan, Mahaabaad	1650	1999	267
Ae. crassa	Koraasan, East of Bojnoord	1200-1800	2000	68
Ae. crassa	Markazi, Tafresh	2020	2000	57
Ae. crassa	Kohkiloye & Boyer Ahmad, Yasouj	2050	2000	4
Ae. crassa	Lurestan, Aligoudarz	2090	2000	41
Ae. crassa	Lurestan, Khoram abad	1140	1999, 2000	42
Ae. cylindrca Host. (2n = 28)	West of Azerbayijan, Khoy-Marand	1200-1700	2000	44
Ae. cylindrica	West of Azerbayijan, Sardasht	1650	2000	45
Ae. cylindrica	Kermanshah	1720	2000	46
Ae. triuncialis L. (2n = 28)	Tehran, Damavand- Gilavand	1950	2000	75
Ae. triuncialis	West of Azerbayijan, Sardasht	1650	2000	218
Ae. triuncialis	Tehran, Delijan	1850	2000	76
Ae. triuncialis	Lurestan, Boroujerd	1140	2000	25
Ae. triuncialis	West of Azerbayijan, Khoy- Marand	1200-1700	2000	219
Ae. triuncialis	15 km from Tehran	1950	2000	77
Ae. triuncialis	Korasan, West of Bojnourd	1200-1800	2000	69

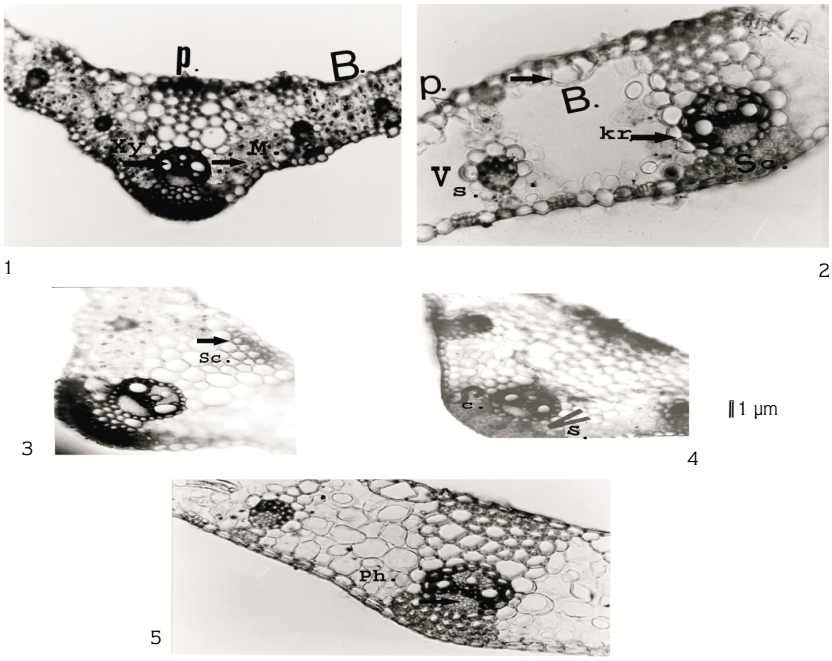

Figure 2. Transections of leaves among the *Aegilops* species: 1) *Ae. speltoides*; 2) *Ae. crassa*; 3) *Ae. columnaris* [X200]; 4) *Ae. tauschii*; 5) *Ae. triuncialis*. [X100]. Sc.: Sclerenchyma strand, B.: Bulliform cells, P.: Prickle, Kr.: Kranz sheath, Ph.: Phloem, Xy.: Xylem, Vs.: Vascular bundle, M.: Mesophyll (parenchyma cells).

Table 2. Matrix of quantitative anatomical characters (leaf and epidermis) and the accessions of the *Aegilops* species.

Characters / Species	W. Xy.	W. Ph	L. St. Ad.	W. St. Ad.	M. N. L.C. Ad.	M. N. Sh. C. Ad.	L. St. Ab.	W. St. Ab.	M. N. L. C. Ab.	M. N. Sh. C Ab.	M. N. St. Ab.	M. N. St. Ad.
Ae. triuncialis (2n = 28)	17	34	50.66	11.22	273.7	24.10	55.08	13.26	232.1	17.68	20.72	14.96
Ae. tauschii (2n = 14)	20.4	17	45.73	9.1	162.24	16.04	35.36	27.6	197.2	14.62	36.4	48.76
Ae. speltoides (2n = 14)	30.6	27.2	35.8	9.52	162.5	-	45.9	10.2	244.8	40.02	36.4	48.7
Ae. cylindrica (2n = 28)	20.4	27.2	50.32	7.82	790.74	28.56	48.28	9.52	209.4	27.2	21.6	27.7
Ae. columnaris (2n = 28)	27.2	58.7	55.7	27.8	158.1	36.89	28.11	11.22	172.3	-	22.84	12.96
Ae. umbellulata (2n = 14)	20.4	23.8	47.6	8.5	206.4	31.8	40.8	10.2	207.7	30.26	22.2	34.75
Ae. crassa (2n = 28)	17	37.4	60.18	12.58	170.3	20.74	52.7	10.8	201.6	16.55	25.2	20.37

W. Xy.: width of xylem, W. Ph.: width of phloem, L. St. Ad.: length of stomata in adaxial epidermis, W. St. Ad.: width of stomata in adaxial epidermis, N. M. L. C. Ad.: mean number of long cells in adaxial epidermis, N. M. Sh. C. Ad.: mean number of short cells in adaxial epidermis , L. St. Ab.: length of stomata in abaxial epidermis, W. St. Ab.: width of stomata in abaxial epidermis, N. M. L. C. Ab.: mean number of long cells in abaxial epidermis, N. M. Sh. C. Ab.: mean number of short cells in abaxial epidermis, N. M. St. A.d.: mean number of stomata in adaxial epidermis, N. M. St. Ab.: mean number of stomata in abaxial epidermis.

Table 3. Matrix of qualitative anatomical characters (epidermis) and the accessions of the *Aegilops* species.

Characters / Species	H. Ad.	H. Ab	P. Ad	P. Ab	P. S. Ep.	W. Sc.	W. Kr.
Ae. triuncialis	+	+	-	+	+	++	++
Ae. tauschii	+	+	+	+	+	++	++
Ae. speltoides	+	-	-	-	+	--	+-
Ae. cylindrica	-	-	-	-	-	--	--
Ae. columnaris	-	-	-	-	-	++	+-
Ae. umbellulata	+	+, -	+	+	-	--	--
Ae. crassa	+	+	+	-	+	++	++

H. Ad.: the occurrence of hair in adaxial epidermis, H. Ab.: the occurrence of hair in abaxial epidermis, P. Ad.: the occurrence of prickle in adaxial epidermis, P. Ab.: the occurrence of prickle in abaxial epidermis, P. S. Ep.: the occurrence of prickle in side of epidermis, W. Sc.: the width of sclerenchyma in epidermis, W. Kr.: the width of Kranz sheath, +: with, -: without, ++: more, --: less, +-: more or less.

(south-east and north-west) and *Ae. cylindrica* (west and north-west) are 2 species that did not have hairs and prickles on their epidermal surfaces (Figure 3 and Table 3).

The Kranz sheaths of *Ae. tauschii*, *Ae. triuncialis*, and *Ae. crassa* were the widest in the semiarid and dry regions (Table 3 and Figure 2) and most of the *Aegilops* species had extensive and thick sclerenchyma, especially *Ae. triuncialis* and *Ae. tauschii* (Table 3 and Figure 2).

The S.D. and C.V. were estimated by the anatomical characters (Table 6a). The largest S.D. was related to the number of long cells in the adaxial epidermis (231.09) (Table 6a) and the smallest S.D. was for the width of the xylem (5.14) (Table 6a). Therefore, the highest C.V. observed was related to the number of long cells in the adaxial epidermis (0.84) and the lowest was related to the number of long cells in the abaxial epidermis (0.11)

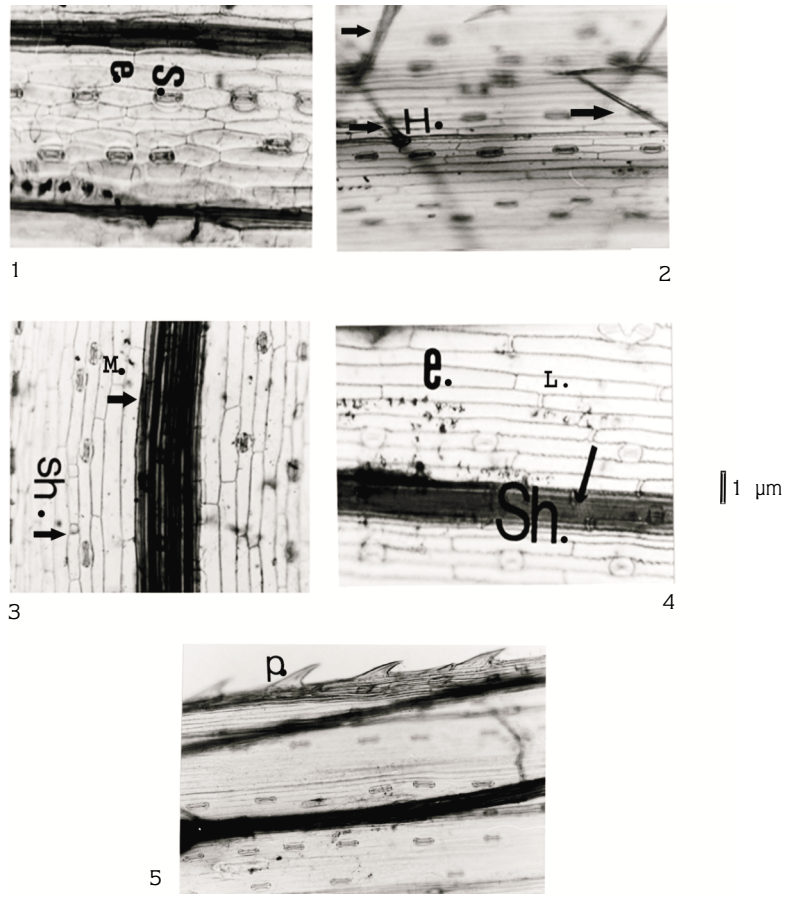

Figure 3. The epidermis of leaves among the *Aegilops* species: 1) *Ae. columnaris*, adaxial epidermis; 2) *Ae. tauschii*, adaxial epidermis; 3) *Ae. cylindrica*, adaxial epidermis; 4) *Ae. tauschii*, abaxial epidermis; 5) *Ae. triuncialis*, abaxial epidermis [X100]. e.: epidermis cell, L.: long cell, Sh.: short cell, H.: hair, S.: stomata, P.: prickle, M.: midrib.

Table 4. Quantitative anatomical characters (leaf and epidermis) among the *Aegilops* species.

1- The width of the xylem	7- The length of stomata in the abaxial epidermis
2- The width of the phloem	8- The width of stomata in the abaxial epidermis
3- The length of stomata in the adaxial epidermis	9- The mean number of long cells in the abaxial epidermis
4- The width of stomata in the adaxial epidermis	10- The mean number of short cells in the abaxial epidermis
5- The mean number of long cells in the adaxial epidermis	11- The mean number of stomata in the adaxial epidermis
6- The mean number of short cells in the adaxial epidermis	12- The mean number of stomata in the abaxial epidermis

Table 5. States of qualitative anatomical characters (epidermis) among the *Aegilops* species.

1- The occurrence of hair in the adaxial epidermis	+: With Hair -: Without Hair
2- The occurrence of hair in the abaxial epidermis	+: With Hair -: Without Hair
3- The occurrence of prickle in the adaxial epidermis	+: With Prickle -: Without Prickle
4- The occurrence of prickle in the abaxial epidermis	+: With Prickle -: Without Prickle
5- The occurrence of prickle inside the epidermis	+: With Prickle -: Without Prickle
6- The width of sclerenchyma in the epidermis	++: More - -: Less
7- The width of the Kranz sheath	++: More - -: Less + -: More or less

Table 6a. Descriptive analysis of the quantitative anatomical characters (leaf and epidermis) among the *Aegilops* species.

Characters	W. Xy.	W. Ph	L. St. Ad.	W. St. Ad.	N. L. C. Ad.	N. Sh. C. Ab.	L. St. Ad.	W. St. Ab.	N. L. C. Ab.	N. Sh. C Ab.	N. St. Ab.	N. St. Ad.
Mean	21.8	32.05	49.42	12.38	274.9	22.5	43.7	13.25	209.3	20.8	24.3	26.9
S.D.	5.14	13.1	7.75	7.02	231.09	12.3	9.63	6.43	23.6	12.9	5.49	12.16
C.V.	0.23	0.4	0.15	0.57	0.84	0.54	0.22	0.48	0.11	0.62	0.22	0.45

S.D.: standard deviation, C.V.: coefficient variation

Table 6b. Descriptive analysis among the accessions of the *Aegilops* species.

Species	Mean	S.D.	C.V.
Ae. triuncialis	60.7	82.59	136.06
Ae. tauschii	52.53	61.16	116.42
Ae. speltoides	57.6	71.9	124.8
Ae. cylindrica	105.47	22.49	210.9
Ae. columnaris	50.98	56.01	109.8
Ae. umbellulata	57.03	70.98	124.4
Ae. crassa	53.7	65	121

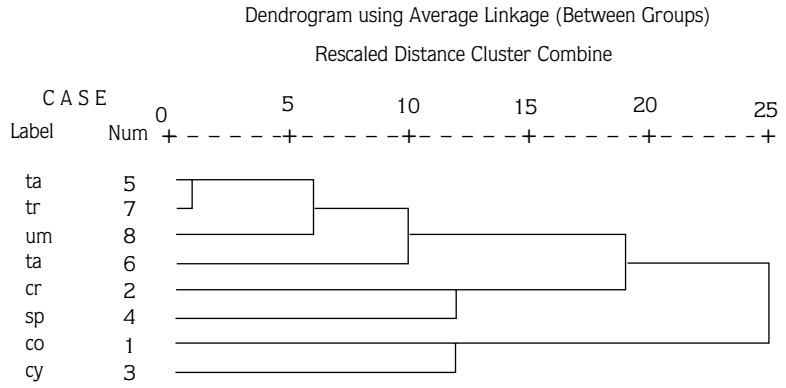

Figure 4. Dendrogram (cluster analysis) of the anatomical characters among the *Aegilops* species (ta: *tauschi* (2n = 14); tr: *triuncialis* (2n = 28); um: *umbellulata* (2n = 14); cr: *crassa* (2n = 28); sp: *speltoides* (2n = 14); co: *columnaris* (2n = 28); cy: *cylindrica* (2n = 28)).

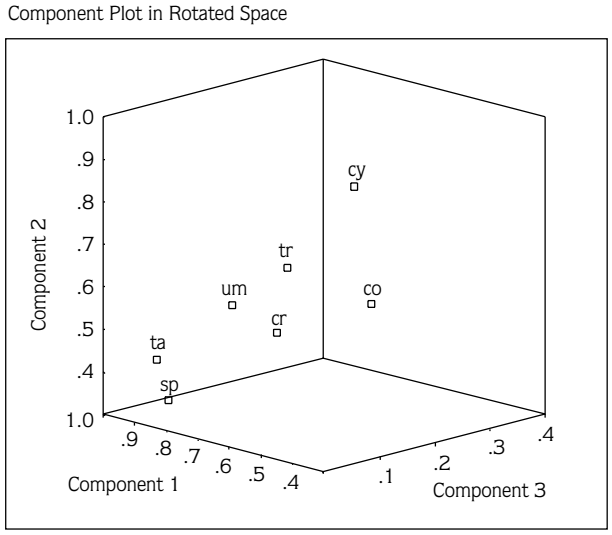

Figure 5. Principle component analysis among the *Aegilops* species (ta: *tauschi* (2n = 14); tr: *triuncialis* (2n = 28); um: *umbellulata* (2n = 14); cr: *crassa* (2n = 28); sp: *speltoides* (2n = 14); co: *columnaris* (2n = 28); cy: *cylindrica* (2n = 28)).

(Table 6a). *Ae. cylindrica* and *Ae. triuncialis* had the highest C.V.s (210.9 and 136.06, respectively) (Table 6b). Therefore, most of the tetraploid species had higher variation of the above-mentioned characters than the diploid species.

The discriminate analysis showed that only 7 quantitative anatomical characters (among 12 quantitative anatomical characters) were significantly different (Sig. 5%) so as to separate these species (Table 7). These species have been precisely differentiated according to these 7 quantitative characters.

In the cluster analysis of the 7 quantitative anatomical characters, 2 groups were distinguished: *Ae. tauschii*, *Ae. umbellulata* Zhuk., *Ae. triuncialis*, *Ae. speltoides*, and *A. crassa* were grouped together, whereas *Ae. cylindrica* and *Ae. columnaris* were different from the other species (Figure 4). The PCA also showed the formation of these 2 groups. In addition, this plot was similar to the results of the cluster analysis (Figure 5). Meanwhile, most of the diploid and tetraploid species were grouped together (Figures 4 and 5).

Discussion

Based on the results of this study, it was observed that some of the anatomical characters of the leaves and epidermis vary with the environment. These variations were related to the cold and warm areas of Iran. Likewise, most of the adaptations among the accessions occurred in the north-west, west, and central areas, particularly those adaptations related to the width of the sclerenchyma. The greatest variations were linked to the width of the phloem, the width of stomata, the mean number of long cells in the adaxial epidermis, the mean number of short cells in the abaxial epidermis, the mean

Table 7. Discriminate analysis of the quantitative anatomical characters among the *Aegilops* species.

Characters	Wilks' Lambda	Sig. (5%)
L. H.	0.715	0.432
L.P.Ad.	0.427	0.119
L.P.Ab.	0.907	0.783
N.S.Ad.	0.800	0.572
N.S.Ab.	0.873	0.712
W.Sc.Ad.	0.875	0.716
W.Sc.Ab.	0.875	0.716

L. H.: length of hair; L. P. Ad.: length of prickle in adaxial epidermis; L. P. Ab.: length of prickle in abaxial epidermis; N. S. Ad.: number of stomata in adaxial epidermis; N. S. Ab.: number of stomata in abaxial epidermis W. Sc. Ad.: width of sclerenchyma in adaxial epidermis; W. Sc. Ab: width of sclerenchyma in abaxial epidermis.

number of stomata in the abaxial epidermis, and the occurrence of prickles in the adaxial epidermis. Hardy et al. (1995) also found that the properties of stomata varied in tropical and subtropical regions. The sclerenchyma was widest in tropical, sub-tropical, and dry areas (*Ae. crassa, Ae. columnaris, Ae. tauschii* and *Ae. triuncialis*) (Figure 2). In addition, Ogundipe & Olatunji (1992) reported that among the taxa of Poaceae the width of the sclerenchyma in the adaxial and abaxial epidermis increased in tropical regions. These distributions, along with the vascular tissues, are very important for determining ethnic variation. Obviously, these species have adapted to different ecological conditions. According to the results of this study, *Ae. triuncialis* (tetraploid species), *Ae. crassa* (tetraploid species), and *Ae. cylindrica* (tetraploid species) were extensively distributed and had the greatest variation. In contrast, the anatomical characters of *Ae. tauschii* (diploid species) showed the least variation among the different regions of Iran. The evidence showed that the tetraploid species were more adapted than the diploid species, but the measures of these characters (e.g., the number of stomata) in diploid species were greater than those of the tetraploid species, which is in agreement with the evaluations by Kaminski et al. (1990), Salomon & Lu (1992), and Slageren (1994). In addition, based on the results reported by Keshavarzi & Rahiminejad (2003), some of the diploid species seem to have a pure genome because they lack gene flow resulting from the lower variation among the diploid species. Meanwhile, the number of vascular bundles and the form of transections of the leaves did not vary among the species, which contradicts Dube & Morisset's studies (1987).

Taxonomically, the best characters that can differentiate *Aegilops* species are as follows: the indumentum and length of hair in the epidermis, the frequency and length of prickles in the epidermis, the width of the sclerenchyma strand, and the number of stomata (Table 7). These characters show the taxonomic position of *Aegilops* species. In addition, these results are closely related to the studies by Dube & Morisset (1987), Jarves & Barkworth (1992), and Masci et al. (1992). Notably, based on the results of discriminate analysis, these characters have significant differences (5%) among these species.

While using genome analysis, Lange & Jochemsen (1992a, 1992b) proved that *Ae. triuncialis* was related to *Ae. umbellulata*, *Ae. tauschii*, and *Ae. speltoides*; similar results were obtained with cluster analysis in the present study (Figure 4). Furthermore, even the genomic formulae of *Ae. tauschii* (DD) and *Ae. crassa* (DDMM) show their similarity (Figure 4) and support a close relationship between the 2 species (Keshavarzi & Rahiminejad, 2003). Cluster and PC analyses showed that some of the diploid and tetraploid species have high gene flow, which refers to their taxonomic complexes; the anatomical characters can resolve these complexes. These 7 anatomical characters differentiated 2 species, *Ae. columnaris* and *Ae. umbellulata*, which, in previous morphological studies, Bor (1968, 1970) considered similar species, although he was not able to separate them using morphological evidence. Despite high morphological similarity between *Ae. columnaris* and *Ae. triuncialis*, and high morphological similarity between *Ae. tauschii* and *Ae. crassa*, these species are well differentiated by anatomical characters. Moreover, anatomically, *Ae. speltoides* is separated from *Ae. triuncialis*. Therefore, these anatomical characters can be used for the aims of taxonomic studies.

Obviously, in this study, the form of vascular tissues, the occurrence of short and long cells, and the silica cells were not considered as appropriate characters for taxonomic ranks, which is in contrast with the reports by Heywood & Moore (1984) and Dube & Morisset (1987).

Finally, regarding the results of this study, *Aegilops* species are becoming increasingly important as potential sources of valuable traits that can be used in wheat (*T. aestivum*) breeding (Bultynck et al., 2003). On the other hand, the tetraploid species are a tolerant species that have evidently environmentally adapted (Anderson, 1993).

This is evident from the highly variable taxa belonging to *Aegilops*, which occur in different ecological and climactic conditions in Iran. Finally, the anatomical characters used in this study seem to be the best for resolving taxonomic issues.

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